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Effect of individual protozoa, *Isotricha intestinalis* and *Metadinium medium,* on ruminal fermentation and methane production *in vitro*[*]

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ABSTRACT

The effect of rumen protozoa *I. intestinalis* and *M. medium* on the fermentation of lucerne hay and maize grain substrates was evaluated *in vitro* using rumen fluid from monofaunated wethers. *I. intestinalis* had a positive effect on the extent of feed degradation, especially in maize-containing substrates, but this effect was offset by an increase in methane production as compared to fauna-free or mixed-fauna fermentations. *M. medium* had no major influence on substrate degradation or production of fermentation products. The fermentation ability of both species, however, was differently influenced by the presence of fauna-free rumen fluid originated from defaunated or faunated animals.

KEY WORDS: rumen, protozoa, fermentation, methane, *Isotricha intestinalis, Metadinium medium*

INTRODUCTION

Mixed rumen protozoa are positively associated with increases in feed degradation and methane production. However, the contribution of individual protozoal species is less known. The fermentation characteristics and methane production of two metabolically different protozoa, *I. intestinalis* and *M. medium*, were evaluated *in vitro*.

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MATERIAL AND METHODS

Six wethers fitted with rumen cannulae were used as donors of rumen fluid. Two animals had a regular mixed protozoal population. The other four animals had previously been defaunated following the method of Jouany and Senaud (1979); two of them were kept free of fauna and the remaining two animals were each inoculated with *I. intestinalis* or *M. medium*. These animals were kept defaunated or monofaunated for more than one year. All animals were fed a maintenance diet twice daily.

Experiment 1

In a first experiment, rumen fluid from defaunated, monofaunated, and faunated animals was used to ferment lucerne hay or maize grain. Whole rumen contents were collected before the morning feeding and strained through a polyester monofilament fabric (250 μm mesh aperture) under a stream of CO_2 to remove solids. Rumen fluids were mixed in a 1:3 ratio with an anaerobic buffer solution (Goering and Van Soest, 1970) kept at 39°C under O_2-free CO_2 gas. This rumen fluid-buffer mixture was utilized immediately to inoculate fermentation vials containing lucerne hay or maize (300 mg) as substrates and incubated anaerobically at 39°C for up to 24 h. Vials without substrate were used as controls. At the end of the incubation period gas production was measured with the aid of a pressure transducer and a sample collected for analysis of constituents by gas chromatography. Vial contents were centrifuged; supernatants were processed for analysis of soluble fermentation products and pellets used for estimation of dry matter degradation (DMD). The experiment was repeated in time.

Experiment 2

Experiment 2 was designed to minimize the confounding effect of donor animal on the influence of fermentation activities by protozoa. Rumen fluid from defaunated, monofaunated, and faunated animals was obtained as above for Experiment 1. *I. intestinalis* and *M. medium* cells were collected by a low-speed centrifugation, and then resuspended in rumen fluid supernatant from defaunated or faunated animals and used to ferment an lucerne:maize (70:30) mixed feed. Rumen fluid from defaunated and faunated animals was also centrifuged and the supernatant and pellet obtained were mixed. Vials without substrate were used as controls. Measurements were as in Experiment 1.

Data was statistically analysed by one-way analysis of variance using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC).

RESULTS AND DISCUSSION

The fermentation characteristics of the protozoal inocula differed depending on the substrate (Table 1). For lucerne hay there was no difference in DMD among inocula. However, in monofaunated inocula, gas production decreased while total VFA production increased as compared to fauna-free and mixed fauna treatments. This favourable shift in the partition of fermented organic matter between VFA and gas in monofaunated inocula could be beneficial to the host in terms of available energy. Unexpectedly, methane production was lower in the mixed fauna inoculum. For the high starch, maize grain substrate, rumen fluid containing *I. intestinalis* was the most efficient inoculum. Increases in DMD ($P<0.05$) were correlated to higher production of gas, methane and VFA (Table 1). However, the amount of VFA and methane produced by unit of substrate disappearance was not different among inocula ($P>0.05$).

Table 1. Fermentation characteristics of rumen fluid from animals harboring single, mixed, and no protozoal species (Experiment 1)

	I. intestinalis	*M. medium*	Fauna-free	Mixed fauna	SEM
Lucerne hay substrate					
DMD, %	55.5	51.9	55.6	52.9	2.99
gas, ml	57.3^{a}	56.6^{a}	65.1^{b}	62.6^{b}	3.64
methane, µmol	706.1^{a}	672.3^{a}	704.8^{a}	596.4^{b}	37.1
VFA, µmol	2256^{a}	2113^{ab}	2074^{b}	1850^{c}	113
Maize grain substrate					
DMD, %	74.6^{a}	66.1^{b}	71.3^{a}	65.4^{b}	2.99
gas, ml	105.3^{a}	89.2^{c}	96.1^{b}	97.2^{b}	3.64
methane, µmol	1017.0^{a}	822.5^{c}	909.4^{b}	835.5^{c}	37.1
VFA, µmol	2916^{a}	2403^{bc}	2490^{b}	2289^{c}	113

^{a,b} whitin a row, means followed by different letters differ ($P<0.05$)

The addition of individual protozoal species to fauna-free rumen fluid from defaunated animals increased the production of methane for *I. intestinalis*, and induced a numerical increase in the amount of DMD for both species as compared to defaunated rumen fluid controls (Table 2). In contrast, total gas and VFA production remained unchanged. When protozoa were combined with fauna-free rumen fluid from conventional animals, the presence of *I. intestinalis* improved substrate degradation markedly without a concomitant increase in methane production. In contrast, *M. medium* presence did not affect DMD but decreased ($P<0.1$) methane emissions.

Table 2. Fermentation characteristics of *I. intestinalis* and *M. medium* suspended in fauna-free rumen fluid from defaunated or faunated animals (Experiment 2)

	I. intestinalis		*M. medium*		Defaunated	Mixed fauna	SEM
	D[1]	F	D	F	D	F	
DMD, %	59.8	68.7 ***	62.1	57.6	57.9	58.2	5.38
Gas, ml	47.9	49.2 *	47.4	51.0	47.2	52.4	2.72
Methane, µmol	581 ***	546	545	504 *	532	532	28.6
VFA, µmol	1584	1714	1689	1633	1688	1740	179

[1] D and F are defaunated and faunated rumen fluid supernatants, respectively; *,*** within a row, means followed by asterisks differ from corresponding controls (*P<0.1 and ***P<0.01)

CONCLUSIONS

The decrease in methane emissions that follows the elimination of protozoa from the rumen was not observed *in vitro* using rumen inocula from animals with a stable microbial population. *I. intestinalis* had a positive effect on the extent of degradation, especially in maize-containing substrates, but this effect was counterbalanced by an increase in methane production. In contrast, *M. medium* did not stimulate methane production. Monofaunation is not an intermediate state between defaunation and faunation; the fermentation profiles of monofaunated rumen fluids were characteristic for each species. The presence of distinct protozoal populations certainly induce changes in the ruminal microbial ecosystem that help to explain the differences observed in this work among inocula, differences that cannot be attributed to protozoa alone.

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